High-Pressure Liquid Chromatographic Determination of Propylthiouracil in Human Plasma

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Received September 11, 1978, from the **Food and Drug Administration, Cincinnati, OH 45202,* and the ‡*College of Pharmacy, University of Cincinnati Medical Center, Cincinnati, OH 45267.* Accepted for publication May 9, 1979.

Abstract □ A high-pressure liquid chromatographic procedure was developed for the determination of propylthiouracil in plasma. The method utilizes a reversed-phase C_{18} chromatographic column and UV detection at 280 nm. The method is sufficiently sensitive for most bioavailability and pharmacokinetic studies. Concentrations between 0.1 and 5.0 μg/ml were measured with a coefficient of variation of 0.6–5.3% for a given day. Methylthiouracil was employed as an internal standard.

Keyphrases □ Propylthiouracil—analysis, high-pressure liquid chromatography, human plasma □ Thyroid inhibitors—propylthiouracil, high-pressure liquid chromatographic analysis, human plasma □ High-pressure liquid chromatography—analysis, propylthiouracil, human plasma

Several methods have been reported for the analysis of the thyrostatic drug propylthiouracil in biological fluids. Each method has some disadvantage with respect to sensitivity, selectivity, reproducibility, and/or adaptability for use in a large-scale bioavailability study. The use of radioactive isotopes (1, 2) is generally unsuitable in bioavailability studies because some modification has to be made in the normal production of the propylthiouracil dosage forms. Existing colorimetric procedures (3, 4) either are relatively insensitive or lack the necessary selectivity to distinguish between the parent drug and metabolites formed by the side-chain oxidation of propylthiouracil (2).

A GLC method (5) was employed in two separate studies in humans (5, 6). The major disadvantages of this procedure are the inherent problems associated with the formulation of derivatives by flash alkylation (7) and the necessity of elevating the column temperature after every two or three injections to remove slowly eluting compounds.

A high-pressure liquid chromatographic (HPLC) procedure using an ion-exchange column was described by Sitar and Hunninghake (8). The procedure was reported to have a lower detection limit of 0.2 μg/ml, comparable to the sensitivity claimed for the GLC procedure (5). Neither the GLC nor HPLC method was considered to be sufficiently sensitive to determine plasma propylthiouracil levels over an 8-hr period following ingestion of a single 150-mg dose.

A specific HPLC method employing an internal standard is presented here. It is both reproducible and sensitive.

EXPERIMENTAL

Reagents and Materials—Propylthiouracil[1] (assayed 101.0%), methylthiouracil[2], reagent grade or equivalent acetic acid, chloroform, methanol, and ammonium sulfate were used as supplied. A stock propylthiouracil solution (60 μg/ml), which also served as a working standard, was prepared in distilled water and stored in the dark at room temperature. Appropriate dilutions were made with water each week to produce additional working standards of 1.2–60 μg/ml. A working standard of the internal standard, methylthiouracil (1.75 μg/ml), was prepared in chloroform and stored in amber glass bottles at room temperature.

Table I—Precision of HPLC Assay of Propylthiouracil for a Given Day [a]

Propylthiouracil, μg/ml	Reproducibility at a Given Concentration: Mean ± *SD*[b], μg/ml	*CV*, %
0.1	0.708 ± 0.037	5.23
0.25	0.676 ± 0.026	3.85
0.5	0.708 ± 0.017	2.40
1.0	0.714 ± 0.011	1.54
2.5	0.724 ± 0.012	1.66
5.0	0.730 ± 0.004	0.55

[a] Six samples were measured at each concentration. [b] Area under the propylthiouracil peak divided by area under the internal standard peak.

Apparatus—A modular high-pressure liquid chromatograph was outfitted with a constant-flow pump[3], a valve-type injector[4], a fixed-wavelength detector[5] employing a 280-nm detector conversion kit[6], an electronic integrator[7], and a stainless steel column, 25 cm long × 3.1 mm i.d., packed with an octadecylsilane reversed-phase support[8]. Absorbance was measured at 280 nm, using a sensitivity of 0.02 absorbance unit full scale (aufs).

Chromatographic Conditions—The mobile phase consisted of deaerated acetic acid–methanol–water (10:75:915). The flow rate was 2.3 ml/min (1500 psi). All determinations were performed at room temperature.

Sample Preparation—Plasma, 3.0 ml, was placed in a suitable silylated (9) glass-stoppered centrifuge tube containing 0.5 g of ammonium sulfate and was mixed vigorously for 1 min. The samples were extracted by mixing for 10 min with 15.0 ml of chloroform, using gentle mechanical agitation. The mixture was centrifuged at 500×*g* for 10 min, and 10.0 ml of the lower layer was transferred to a 15-ml silicon-coated vacuum tube[9] containing 1.0 ml of the internal standard.

The chloroform was evaporated with the aid of a water bath maintained at 60–65° and a gentle air stream. The residue was dissolved in 250 μl of the mobile solvent; the walls of the tube were rinsed with a microsyringe, and the contents were mixed with a vortex mixer. Samples (25 μl) were injected into the chromatograph.

Calibration—Calibration curves were constructed in duplicate for each batch of unknown samples by adding known amounts of propylthiouracil to control plasma. The internal standard was added after the chloroform extraction because preliminary experiments indicated that the methylthiouracil extraction was not reproducible. The propylthiouracil in unknown samples was determined from the calibration curve prepared daily by plotting the average ratio of the area under the peak (*AUPR*) of propylthiouracil to that of the internal standard against the plasma propylthiouracil concentration (micrograms per milliliter). The correlation coefficients exceeded 0.99 for each daily calibration curve. Normalized *AUPR* values were determined by dividing the *AUPR* for each standard by the plasma propylthiouracil concentration (micrograms per milliliter).

[1] Lot 86328, ICN-K&K Laboratories, Irvine, Calif.
[2] ICN Pharmaceuticals, Plainview, N.J.
[3] Model 6000, Waters Associates, Milford, Mass.
[4] Universal injector model, Waters Associates, Milford, Mass.
[5] Model 202/401, Waters Associates, Milford, Mass.
[6] Waters Associates, Milford, Mass.
[7] Model 3380A, Hewlett-Packard, Avondale, Pa.
[8] Spherisorb 10 μ, Spectra-Physics, Santa Clara, Calif.
[9] Red stopper vacuum tube, Becton-Dickinson, Rutherford, N.J.

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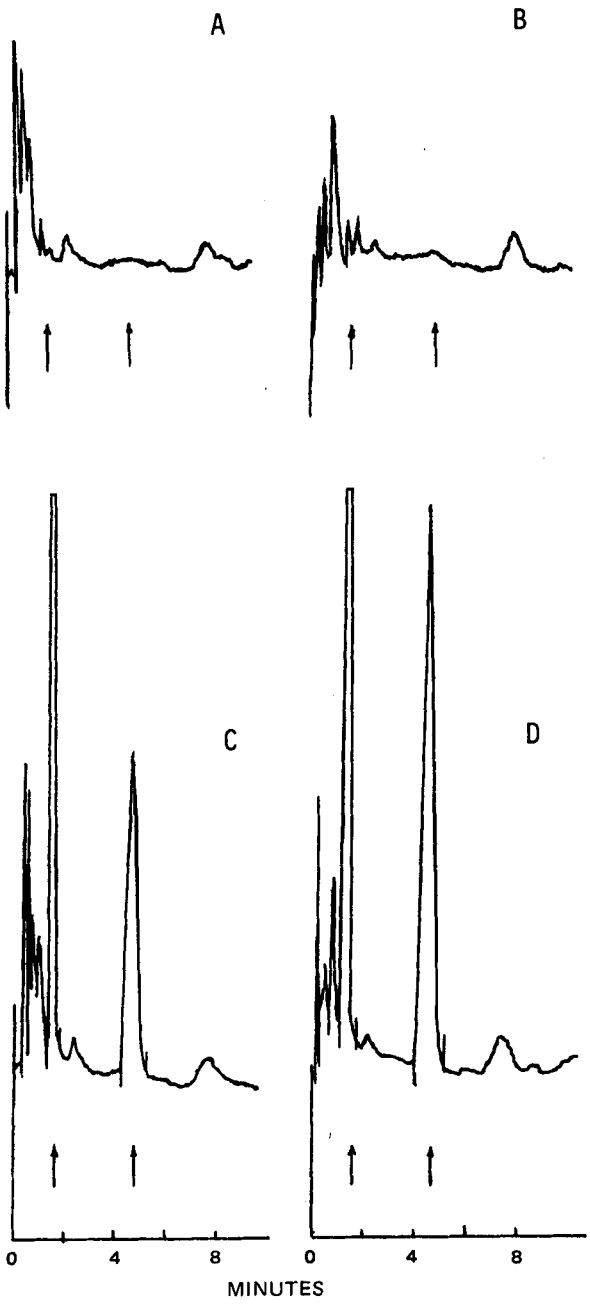

Figure 1—*HPLC determination of propylthiouracil in human plasma. Key: A, chromatogram obtained when 3 ml of control plasma was extracted and the absorbance was measured at 254 nm; B, chromatogram of the same control plasma with the absorbance measured at 280 nm; C, representative chromatogram of plasma containing propylthiouracil and the internal standard with the absorbance measured at 254 nm; and D, chromatogram of the same plasma sample containing propylthiouracil and the internal standard with the absorbance measured at 280 nm. The arrows refer to the retention times of propylthiouracil (4.8 min) and the internal standard (1.5 min).*

RESULTS AND DISCUSSION

A selective method for measuring propylthiouracil in plasma at suitably low concentrations was desired. Because of the large numbers of plasma samples expected in a planned bioavailability study (10), the time required for each analysis was critical. Because of its relative ease of operation and sensitivity, HPLC appeared to represent the best approach. A reversed-phase microparticulate column was found to be superior to the ion-exchange column used by Sitar and Hunninghake (8) because of reduced analytical times, greater peak sharpness, and less baseline noise.

The chloroform extraction of propylthiouracil is similar to that described by Sitar and Hunninghake (8). In an effort to improve the extraction efficiency, the pH of the plasma was lowered before the chloroform extraction. Results were negative and were attributed to the coagulation of protein material, which hindered extraction of propylthiouracil that may have been occluded. The addition of ammonium sulfate has been demonstrated to have a positive effect on the propylthiouracil extraction efficiency (5).

Irreversible propylthiouracil adsorption to the surface of laboratory glassware was encountered in preliminary recovery studies, particularly during the evaporation of the chloroform extract. As a result, silylated or silicon-treated glassware was used.

Chromatograms obtained in the analysis of control human plasma and human plasma from a volunteer who had received a single propylthiouracil dose are shown in Fig. 1. The chromatogram of control human plasma demonstrated no interfering peaks. Sensitivity was increased approximately twofold when propylthiouracil was measured at 280 nm instead of 254 nm, with no significant increase in the area of the extraneous peaks or background noise.

While the chromatographic methods (5, 8) probably measure only unchanged propylthiouracil, their selectivity was not specifically investigated. The effect of known propylthiouracil metabolites on the assay was evaluated by adding propyluracil (11), *S*-methylpropylthiouracil (11), propylthiouracil glucuronide (11–14), the sulfinic and sulfonic acid derivatives of propylthiouracil (15), and sulfate (11) to plasma samples, which were then analyzed.

Propyluracil exhibited a retention time that did not interfere with the measurement of propylthiouracil or the internal standard peaks. *S*-Methylpropylthiouracil was extracted but failed to elute from the column under the described conditions. The sulfinic and sulfonic acid derivatives, propylthiouracil glucuronide, and sulfate were not extracted from the aqueous layer by chloroform. Since none of the known metabolites interfered with methylthiouracil or propylthiouracil, the method is probably specific for propylthiouracil.

The precision or reproducibility of the method for a given day is shown in Table I using normalized data. Propylthiouracil stability in plasma was assessed also. Control plasma was spiked with standard propylthiouracil solutions, and the samples were frozen. On Days 1–4 and 8, duplicates of each standard concentration were thawed and analyzed. No significant differences were observed.

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ACKNOWLEDGMENTS

Supported by the Food and Drug Administration under the SARAP Program.

The authors thank Dr. Raymond H. Lindsay, Director of Pharmaceutical Research, Veterans Administration Hospital, Birmingham, Ala., for generously supplying propylthiouracil glucuronide, propylthiouracil sulfinic acid, and propylthiouracil sulfonic acid.

Ion-Pair Reversed-Phase High-Pressure Liquid Chromatography of Cough–Cold Syrups I: Pseudoephedrine Hydrochloride, Brompheniramine Maleate, and Dextromethorphan Hydrobromide

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Received March 23, 1979, from *Parke-Davis, Division of Warner-Lambert Company, Morris Plains, NJ 07950.* Accepted for publication May 14, 1979.

Abstract □ Pseudoephedrine hydrochloride (I), brompheniramine maleate (II), and dextromethorphan hydrobromide (III) in a cough–cold syrup were separated and determined by ion-pair reversed-phase high-pressure liquid chromatography. The separation was carried out using a μBondapak C_{18} column (30 cm × 3.9 mm i.d.) and a mobile phase of acetonitrile–water–acetic acid (40:60:1) with 0.01 *N* 1-octanesulfonic acid sodium salt and 0.05 *N* potassium nitrate. Detection was accomplished using a UV detector at 265 nm for I and II; III was monitored at 280 nm. Concentration *versus* peak height plots in the ranges of 0.37–1.9 mg/ml for I, 0.025–0.126 mg/ml for II, and 0.125–0.625 mg/ml for III were linear. Ten consecutive injections of a mixture gave a percent relative standard deviation of <1% for all three components. Average recoveries from laboratory-prepared samples were 100.5% for I, 100.9% for II, and 100.1% for III. No precolumn cleanup was necessary, and the chromatogram was complete in 16 min.

Keyphrases □ Cough–cold syrup—analysis, ion-pair reversed-phase high-pressure liquid chromatography, pseudoephedrine hydrochloride, brompheniramine maleate, dextromethorphan hydrobromide □ Pseudoephedrine hydrochloride—analysis, ion-pair reversed-phase high-pressure liquid chromatography, cough–cold syrups □ Brompheniramine maleate—analysis, ion-pair reversed-phase high-pressure liquid chromatography, cough–cold syrups □ Dextromethorphan hydrobromide—analysis, ion-pair reversed-phase high-pressure liquid chromatography, cough–cold syrups

Antihistamines, antitussives, and decongestants are used extensively in cough–cold syrups. Often, two or more of these compounds are combined in a preparation, and an isolation of the desired analyte from the other components is necessary prior to measurement.

Reversed-phase high-pressure liquid chromatography (HPLC) was used to investigate 21 antihistaminic, antitussive, and analgesic drugs in cough–cold mixtures (1), and the separation of four antihistamines was examined by reversed-phase HPLC (2). Ion-pair reversed-phase HPLC is a relatively new and extremely useful technique. General discussions of the method were published (3, 4).

The present study investigated the feasibility of applying ion-pair reversed-phase HPLC to the separation and assay of a mixture of antihistaminic, antitussive, and decongestant drugs in a cough–cold syrup.

EXPERIMENTAL

Apparatus—A piston pump[1], an automatic sampler[2], and two detectors (one set at 280 nm[3] and the other at 265 nm[4]) were used. The chromatograms were recorded on a two-pen recorder[5].

Column—A bonded reversed-phase C_{18} column[6] was used.

Samples—A cough–cold syrup was prepared so that each 5 ml contained 30 mg of pseudoephedrine hydrochloride (I), 2 mg of brompheniramine maleate (II), 10 mg of dextromethorphan hydrobromide (III), and 5% alcohol. The standard solution was prepared by dissolving 120 mg of I[7], 8 mg of II[8], and 40 mg of III[7] in water and diluting to 100.0 ml. The sample solution was prepared by transferring 5 ml of the cough–cold syrup quantitatively to a 25-ml volumetric flask and diluting to volume with water.

Analysis—The chromatographic conditions were: flow rate, 0.8 ml/min (700 psi); mobile phase, acetonitrile[9]–water–acetic acid (40:60:1) with 0.01 *N* 1-octanesulfonic acid sodium salt[10] and 0.05 *N* potassium nitrate[11]; temperature, 25°; and detector scale, 0.064 aufs for 280 nm and 0.1 aufs for 265 nm.

Ten-microliter aliquots of standard and sample solutions were injected in duplicate onto the column using the chromatographic conditions.

Table I—Accuracy Study for HPLC Assay of Pseudoephedrine Hydrochloride (I), Brompheniramine Maleate (II), and Dextromethorphan Hydrobromide (III) from Aqueous Solutions

Percent of Target	Recovery, % I	II	III
85.0	100.6	99.9	102.0
90.0	98.4	97.2	100.3
95.0	102.0	101.8	100.9
97.0	101.4	102.1	99.2
99.0	100.6	98.8	99.8
101.0	100.0	98.8	99.8
103.0	100.0	100.0	100.0
105.0	98.9	98.4	99.1
110.0	100.7	100.9	100.0
115.0	98.3	101.3	100.1
Average	100.1	99.9	100.1
Range	98.3–102.0	97.2–102.1	99.1–102.0
RSD	±1.2	±1.6	±0.84

DISCUSSION

Mobile Phase Selection—Methanol and water or acetonitrile and water mixtures in various proportions were tested with no satisfactory separation of I–III. Sulfonic acid sodium salts of 1-butane, 1-pentane, 1-hexane, and 1-octane and dioctylsulfosuccinate sodium salt[7] (IV) were

[1] Milton Roy.
[2] DuPont 834.
[3] Laboratory Data Control model 1203.
[4] Perkin-Elmer LC 55.
[5] Linear recorder 385.
[6] μBondapak C_{18} column, Waters Associates.
[7] NF reference standard.
[8] USP reference standard.
[9] Burdick & Jackson.
[10] Eastman Organic Chemicals.
[11] Baker Analyzed Reagent.

0022-3549/79/1100-1463$01.00/0